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Estimation of Concentration of Selected Heavy Metals in Muscle, Liver and Bones of Pigs

R. Thomas*, S. Singha, M. Saikia, R. Kalita, Z. Baruah and N. Saharia

Food Quality Control Laboratory, ICAR-National Research Centre on Pig,

Rani, Guwahati, 781 131, Assam, India

ABSTRACT

Pork is a very rich and convenient source of nutrients including microelements. Evaluation and monitoring of heavy metal concentration in pork is very much important from the consumer safety point. In the present study, concentration of selected heavy metals viz., Tin (Sn), Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) in pig muscle, liver and bones collected from different parts of Kamrup District, Assam were evaluated using atomic absorption spectroscopy. The results indicated that the concentration of studied metals was not exceeding the maximum permissible limit (MRL) recommended by FSSAI. The results also indicated that the concentration of Sn was significantly higher in bones (14.52±2.41ppm) compared to muscle tissues (9.53±2.49 ppm). The concentration of Sn, Pb, Cd, As and Hg in the muscle tissue was in the range of 0.00-21.61 (ppm), 0.01-0.07 (ppm), 0.00-0.005 (ppm), 0.00-1.75 (ppb) and 0.00-0.40 (ppb), respectively. The mean concentration of Cd and As in muscle tissue (0.001±0.001ppm and 0.85±0.28ppb, respectively) was comparatively lower than that in the liver (0.01± 0.01ppm and 1.13±0.81ppb, respectively). The results also indicated that the level of Pb was more in muscle tissues compared to liver. In liver, the range of heavy metals were 0.00-0.04 (ppm), 0.00-0.03 (ppm) and 0.00- 2.31 (ppb) for Pb, Cd and As, respectively.

Keywords: *Heavy metals, Atomic Absorption Spectroscopy, Muscle tissue, Pig, Risk assessment*

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INTRODUCTION

Heavy metals are naturally present in the environment but their occurrence has progressively been increasing with the increase in industrialization. In many countries, industries with improper waste disposal and management and urban activities have generated global health concerns due to the risks of heavy metals ending up in the food chains. Naturally, metals with a density of more than 5 mg cm-3 are considered heavy metals, and it generally includes elements such as arsenic, cadmium, chromium, copper, lead, nickel, molybdenum, vanadium and aluminium, other rare metals (Das and Das 2018). Humans are exposed to heavy metals mostly by ingestion and inhalation. Presence of heavy metal in high concentration was reported in water, soil and fodder from areas closer to industry (Singh *et al.*, 2019).

Globally, meat is considered to be a very rich and convenient source of protein, vitamin B, microelements, fatty acid and cholesterol (Chowdhury *et al.*, 2017). However, in many countries, especially in developing and underdeveloped countries, the livestock production system is at high risk of contamination with toxic metals. The heavy metal contaminants enter livestock production systems mostly as a result of polluted air, water, soil, and consumption of contaminated feed. Most of these contaminants have no known metabolic function, but when present in the body, they disrupt many normal cellular processes, leading to toxicity in a number of organs. Most of these elements have the tendency to get accumulate in different body tissues, viz. muscle, bone, liver etc. of animals, which in turn will enter into the human food chain. A series of adverse effects on human metabolism has reported from exposure to heavy metals (Rajaganapathy *et al.*, 2011). For example, organic lead (Pb) compounds can enter the brain giving rise to a toxin in central nervous system. Lead also causes oxidative stress and destroys the oxidant/ antioxidant balance of cells (Patocka, 2008). Moreover, these toxic metals are also seen interacting metabolically with nutritionally essential metals like calcium (Ca), Iron (Fe) and

*Corresponding author E-mail address: thomasrlpt@gmail.com

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Zinc (Zn) (Goyer, 1997). Another well known example is 'Minamata Disease', which is a disease of the central nervous system as a result of mercury poisoning caused by consumption in large amounts of fish and shellfish contaminated with mercury. Only the quality control and assurance system can ensure quality and safe meat which is free of physical, chemical as well as biological hazards (Girish *et al.,* 2017). Therefore discrete efforts need to be given for ensuring quality and safety of meat. There is an imperative need to evaluate heavy metals in meat to confirm the level of contamination. The present study was aimed to assess the concentration of selected heavy metals in muscle, liver and bones of pigs from different parts of Kamrup District, Assam.

MATERIALS AND METHODS

***Collection of samples*:** Edible muscle tissue (ham), liver and bone (femur) of pigs (non-descript, female pigs of 1-1.5 years old were collected from the local markets of Kamrup District, Assam with three repetitions to investigate the heavy metal content. In total, 30 samples of each group (muscle, liver and bone) were collected and the samples were transported to the laboratory in chilled condition. The polypropylene sample containers for carrying the samples were pre-washed in acid solution and rinsed with distilled water twice to ensure minimal interference. After collection, the samples were labeled and preserved by storing at low temperature (-18^{o}C).

***Ashing of samples*:** Before analysis, the stored samples were thawed and washed with distilled water and air dried for 24 h. These were then dried again at 80^{0}C in an oven for 24 h or till it loses its maximum moisture. Separate clean, ceramic mortar pestle was used to grind samples so as to avoid cross-sample contamination. About 2g of those powdered samples were subjected to decarbonization for few minutes in a porcelain crucible. The samples were then ashed in a muffle furnace at about 450^{0}C under a gradual increase (d"50^{0}C/hr) in temperature for about 5 hr until a white or grey ash residue was obtained (AOAC Official Method 999.11, 1999).

***Digestion of samples*:** Two different acids were used for digestion of the sample based on the element, assumptive concentration level and instrument used. In order to detect Tin (Sn), Lead (Pb) and Cadmium (Cd), the samples were digested in 20 ml of 6N HCL. Similarly, 8N HNO_3 was used to detect Arsenic (As) and Mercury (Hg) in ppb level. The digested samples were boiled for 2-3 minutes and then poured down through Whatman filter paper No. 42 in a volumetric flask making up the volume up to 100 ml by de-ionized water.

***Preparation of working standard solution*:** The standard solutions of Tin (AA63N-1), Lead (AA29N-1), Cadmium (AA08N-1), Arsenic (AA03N-1) and Mercury (AA34N-1) were procured from AccuStandard (New Haven, USA) and used for calibration by diluting from 1000μg/ml stock. Double distilled demonized water was used for all dilutions. Determinations of the metal concentration in the samples were carried out based on the calibration curve. For all the elements, three standards were taken to get a standard calibration curve. Stock solution of 100 ppm was prepared by dissolving 4ml of the provided Sn standard solution into 36 ml of de-ionized water. Next, to prepare working solution of 10, 20 and 30 ppm, 4, 8 and 12 ml pipetted out from 100 ppm standard solution into 50 ml centrifuge tube and made the volume up to 40 ml with De-ionized water. Similarly, aliquots of 5, 10 and 15 ppm and 0.5, 1 and 1.5 ppm were prepared as working standard for of Pb and Cd, respectively. Sn, Pb and Cd were estimated in ppm (μl/ml) level using Flame mode of atomic absorption spectrophotometer. As and Hg were estimated in ppb (ng/ml) level using hydride generator atomic absorption spectrophotometer (HG3000). The concentration of standards prepared for As was in the range of, 10, 20 and 30 ppb, while that of Hg was 20, 30 and 40 ppb.

Equipment and apparatus: Atomic Absorption Spectrophotometer (Make: GBC, Australia; Model: Savant AA), equipped with single element hollow cathode lamp, an atomization system consists of nebulizer, spray chamber and acetylene burner was used.

Statistical analysis: The data obtained from AAS analysis were subjected to statistical analysis using SPSS, version 14.0 (SPSS, 2007). Mean values, standard deviation, ranges are reported.

RESULTS AND DISCUSSIONS

The concentrations of different heavy metals viz. Sn, Pb, Cd, As and Hg in muscle tissue, liver and bones of pigs are given in Table 1. The results indicate that Sn and Hg were not present in any of the liver samples tested. Also, Hg was not detected in bone samples. Moreover, their concentrations in muscles were very low and were well within the prescribed MRLs of 2.5, 50, 1.5, 1.1 and 1 ppm for Pb, Sn, Cd, As and Hg, respectively by FSSAI. Hg concentrations in muscle tissue varied from non-detectable to 0.40 ppb. The maximum concentration of Hg found in this study was much lower than that found in Galician pigs in Spain (Lopez-Alonso *et al.,* 2007).

Table 1: Metal concentrations (mean ± SD and range) of different samples

Samples	Sn		Pb		Cd		As		Hg	
	Means ±SD (ppm)	Range (ppm)	Means ±SD (ppm)	Range (ppm)	Means ±SD (ppm)	Range (ppm)	Means ±SD (ppm)	Range (ppm)	Means ±SD (ppm)	Range (ppm)
Muscle (n=10)	9.53 ± 2.49	0.00 - 21.61	0.05 ± 0.02	0.01 - 0.07	0.001 ± 0.001	0.00 - 0.005	0.85 ± 0.28	0.00 - 1.75	0.15 ± 0.08	0.00 - 0.40
Liver (n=10)	Not detected		0.022 ± 0.02	0.00 - 0.04	0.01± 0.007	0.00 - 0.03	1.13 ± 0.81	0.00- 2.31	Not detected	
Bone (n=10)	14.52 ± 2.41	0.00-74.33	0.046 ± 0.03	0.00 - 0.25	0.01 ± 0.001	0.00 - 0.05	Not detected		Not detected	

The concentration of Sn in bone was found to be higher compared to that of muscle tissue. This could be attributed to the fact that, Sn tends to accumulate particularly in the bone and to a lesser extent in the liver, lung, tongue, lymph nodes and kidneys (Howe *et al.,* 2005). However, the values were within the MRL limits specified in FSS (Contaminants, Toxins, and Residues) Regulations, 2011. Khalafalla *et al.* (2016) reported that the concentration of Sn is more in canned meat, rather than in fresh meat. The same authors also reported that Sn levels were ranged from non-detectable to 6.22 ppm and 6.35 ppm for canned chicken luncheon and canned beef luncheon, respectively. However, a higher concentration of Sn was observed in the current study compared to the earlier reports.

The highest Pb concentration (0.05± 0.02ppm) was detected in muscle while the lowest value (0.022±0.02ppm) was detected in liver. The FSSAI has established maximum admissible levels of 2.5ppm for Pb in meat. The possible source of origin of heavy metals in the analyzed meat samples could be the drinking water, as it was reported that a large number of drinking water sources in the Kamrup District, Assam were contaminated with higher concentrations of cadmium, manganese and lead (Chakrabarty *et al.,* 2011). Although obtained results in this study were below the standard permissible levels. Compared to the results reported in previous work (Lopez-Alonso *et al.,* 2007), the Pb residue in muscle tissue was higher compared to that of liver. Lopez-Alonso *et al.* (2007) also reported the mean concentrations of Pb as 0.004 ppm and 0.003 ppm in liver and muscles of Galician pigs, respectively. The current study indicated that Pb accumulates more in muscle and bone compared to liver. Similar result was also reported in chicken (Bayko *et al.,* 1995). Pb residues in the studied samples were higher than those reported in meat of domestic animals in Nigeria (Odoh *et al.,* 2016). In another study, canned luncheon pork showed highest lead residue than other processed product, and was attributed to release of Pb from soldered cans (Santhi *et al.,* 2008). Besides meat, higher concentration of Pb was also reported in rice, red lentil, fish, and chicken in Kolkata, India (Das and Das 2018).

Concentration of Cd in muscle tissue varied from undetectable to 0.005 ppm with a mean value of 0.001±0.001ppm (Table 1). Arsenic concentrations up to 1.75 ppb were detected in muscle tissue. Arsenic concentrations were below the detection limit in all bone samples. In the current study, As residues in the samples were very low than those found in the Galician pigs' muscle and liver i.e. mean conc. of 3 ppb and 13 ppb, respectively (Lopez-Alonso *et al.,* 2007). The mean concentration of Cd and As in muscle tissue was significantly lower than that of the liver. It was reported that toxic metal contents often tend to accumulate in offal products such as liver than in muscles of animal (Lawrie, 2006). Studies also indicated that average concentrations of metals in tissues, especially in the liver and kidneys, depend partly on the age, sex and breed of the animals (Lopez-Alonso et al. 2007). The amount of Cd found in this analysis was much lower than that of reported in pork samples from Nigeria's industrial area (Odoh et al.2016). Cd residue in pig was also found lesser than the common fish available in the industrial and urban cities (Tabouk, Riyadh, Damamm and Jazan) in Kingdom of Saudi Arabia (Alturiqi and Albedair 2012). Santhi *et al.* (2008) studied the Cd contamination in processed pork products such as luncheon meat, ham, salami, sausage etc. in India and reported that Cd has exceeded the maximum permissible limit in 95.83% of the processed products. However, in the present study, both Cd and As concentration was well within the prescribed MRLs by FSSAI.

CONCLUSION

This study was carried out to assess the level of selected heavy metals in muscle tissue, liver and bones of pig. In general, the concentration of Pb, Sn, As, Hg and Cd in the assessed samples were well within the FSSAI prescribed MRLs. However, the study points towards the fact that long term assessment and monitoring of harmful heavy metals in pork, with much robust number of samples, is very much essential from the point of ensuring consumer safety. In addition, appropriate steps need to be taken to prevent heavy metals from entering into the food chain.

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COMPETING INTERESTS

The authors have no competing interests either technical, financial or personal between themselves or others that might bias the work

ETHICAL STATEMENT

Not applicable

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